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Short-term Variability of Repolarization Is Superior to Other Repolarization Parameters in the Evaluation of Diverse Antiarrhythmic Interventions in the Chronic Atrioventricular Block Dog

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Abstract: Short-term variability (STV), to quantify beat-to-beat variability of repolarization, is a surrogate parameter that reliably identifies proarrhythmic risk in preclinical models. Examples include not only the use in the chronic atrioventricular block (CAVB) dog model whereby it was developed but also in vulnerable patients with heart failure or drug-induced long QT syndrome. In the CAVB dog model, STV can specifically distinguish between safe and unsafe drugs in proarrhythmic screening. Conversely, this dog model also offers the possibility to evaluate antiarrhythmic strategies in a setting of Torsades de Pointes (TdP) induction with a standard I_{Kr} inhibitor. The different antiarrhythmic interventions studied in suppression and prevention of drug-induced TdP in vivo in the CAVB dog model and in vitro in canine ventricular cardiomyocytes are described in this overview. We provide evidence that STV predicts the magnitude of antiarrhythmic effect against TdP better than other repolarization parameters in both suppression and prevention conditions. Moreover, suppression and prevention experiments revealed the same level of antiarrhythmic efficacy, whereas cellular experiments seem more sensitive in comparison with drug testing in vivo. Together, these observations suggest that STV could be used as a consistent indicator to rank efficacy of antiarrhythmic interventions in a number of conditions.

Key Words: short-term variability, antiarrhythmic drug, Torsade de Pointes, electrophysiology, animal model

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INTRODUCTION

Life-threatening ventricular arrhythmias, such as ventricular tachycardia and ventricular fibrillation, are responsible for approximately 2 thirds of all sudden cardiac deaths.[1] Several studies have demonstrated that patients affected by acute coronary syndromes and structural heart disease related to ischemic cardiomyopathy show a diminished cardiac function and an enhanced risk for ventricular arrhythmias.[2,3] Although implantation of an internal cardioverter-defibrillator is designed to prevent sudden cardiac death in this patient population,[2,3] this device-based strategy, despite being costly, does not prevent the incidence of ventricular arrhythmias and is associated with a number of shortcomings.[4] After the Cardiac Arrhythmia Suppression Trial, a number of clinical trials have tried to demonstrate the benefit of using antiarrhythmic drug in post-myocardial patients, but only a few drugs gained approval and reached the market because of lack of efficacy and/or proarrhythmic adverse effects.[5] The development of efficacious adjunctive (pharmacological) therapies remains therefore a major challenge for pharmaceutical companies.

In an effort to predict the proarrhythmic vulnerability of patients at risk and torsadogenic properties of drug, several markers have been developed for preclinical and clinical purposes[6] and were derived from the original concept of triangulation, instability, and dispersion of repolarization of Hondeghem et al.[7] In addition, the increase in beat-to-beat variability of repolarization (BVR), which can be quantified as short-term variability of repolarization (STV), associates better to the clinical proarrhythmic susceptibility than the sole prolongation of repolarization in patients affected by heart failure or drug-induced long QT syndrome.[8,9] Repolarization reserve, known as the redundancy in mechanisms allowing a proper repolarization process,[10] is reduced in patients vulnerable to ventricular arrhythmias.[11] By distinguishing between high- and low-risk (heart failure) patients, STV seems to represent a reliable surrogate parameter for the assessment of repolarization reserve.[12]

STV was originally developed in the chronic atrioventricular block (CAVB) dog, a well-characterized animal model with an enhanced susceptibility to Torsade de Pointes (TdP) arrhythmias.[13] In this model, the increase in STV, derived from the left ventricular monophasic action potential duration (LV MAPD), is also superior to repolarization delay in reflecting TdP inducibility.[14] Therefore, the sensitivity and

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specificity of this parameter have also been used in a setting of proarrhythmic screening to confirm drugs' safety[15,16] and proarrhythmic effects.[14,17]

Over the years, the enhanced proarrhythmic susceptibility of the CAVB dog model has been used also for the evaluation of antiarrhythmic interventions, including drugs.[15–17] The purpose of this work is to provide a comprehensive overview of the different antiarrhythmic interventions that have been investigated in vivo in the CAVB dog model and in vitro in isolated canine ventricular cardiomyocytes as published before and to compare their outcomes to deduce general principles on parameters used in antiarrhythmic screening strategies within this model system. Their respective antiarrhythmic efficacies against drug-induced TdP and early afterdepolarizations (EADs) were quantified, ranked, and compared with changes observed in repolarization, STV, and a surrogate for spatial dispersion of repolarization (in vivo only).

Arrhythmogenesis in the CAVB Dog Model

The acute and chronic adaptations of the heart occurring at the contractile and structural levels after ablation of the atrioventricular node have been well described.[13] In addition, electrical remodeling occurs and mainly comprises the

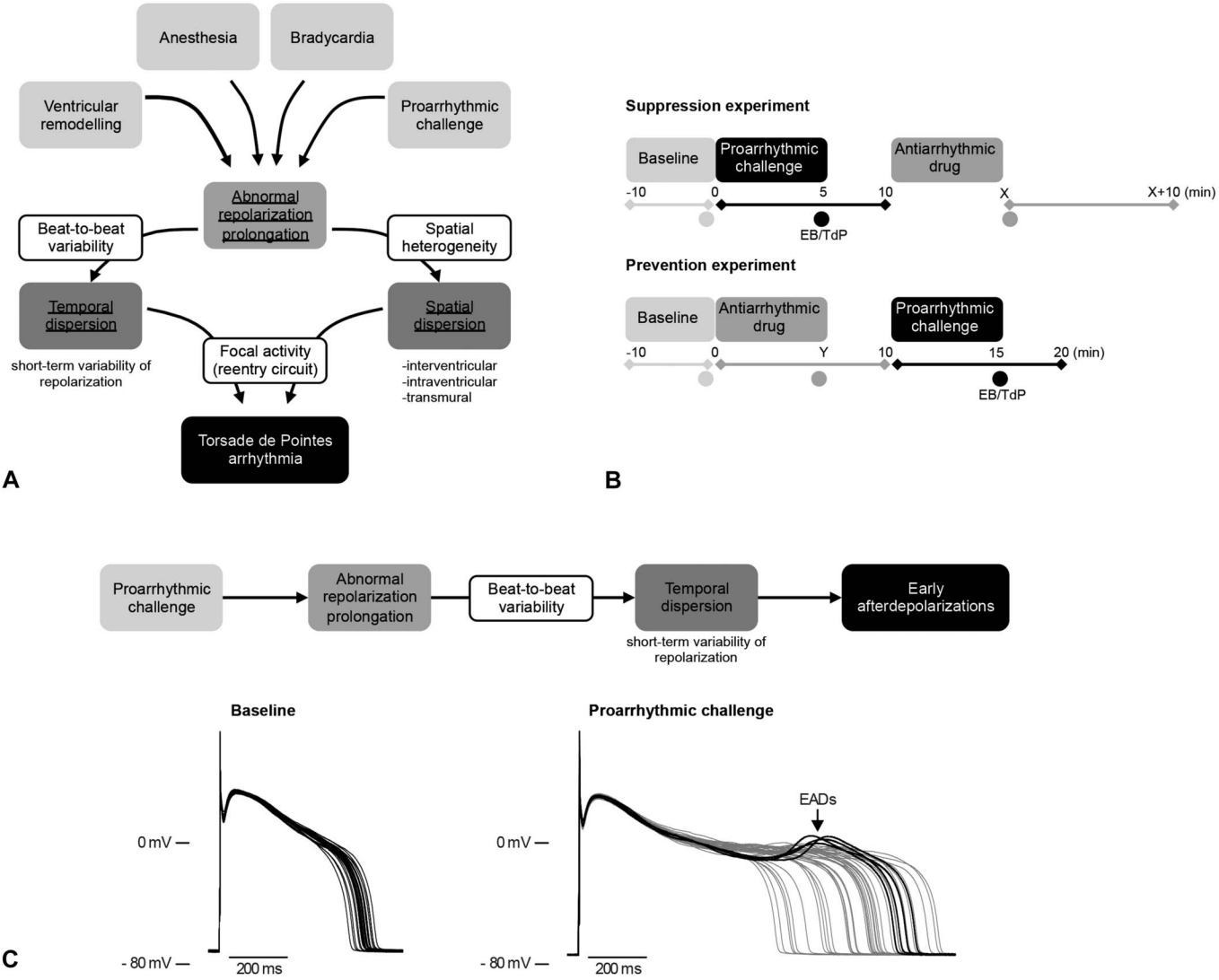

FIGURE 1. A, Arrhythmogenesis in the CAVB dog model. B, Suppression and prevention experimental setups for antiarrhythmic drug evaluation. Lines define intervals during which arrhythmias were monitored to determine TdP inducibility and AS at corresponding periods. Dots define timepoints at which electrophysiological parameters (ECG intervals, MAPD, and STV) were measured. For the proarrhythmic challenge period, they were measured before incidence of the first ectopic beat or (if possible) TdP episode. In the prevention experiments, in the absence of arrhythmias, these parameters were measured at the end of the proarrhythmic challenge infusion (15 minutes). C, Induction of EADs by a proarrhythmic challenge in isolated canine ventricular cardiomyocytes. EADs occur under conditions of increased action potential duration and STV of repolarization. AS, arrhythmia score; MAPD, monophasic action potential duration; STV, short-term variability of repolarization; X, End of antiarrhythmic drug in suppression experiment; Y, end of antiarrhythmic drug in prevention experiment; EB, ectopic beat; TdP, Torsade de Pointes; EADs, early afterdepolarizations.

downregulation of the slow and rapid components of the delayed rectifier outward potassium currents (I_{Ks} and I_{Kr}) along with disturbed calcium handling.[13] Besides ventricular remodeling,[18] also anesthesia[19] and bradycardia[20] predispose approximately 75% of canine hearts to develop TdP on a class III antiarrhythmic (eg, dofetilide or sertindole) challenge (Fig. 1A). All these factors abnormally prolong repolarization and increase its temporal[14] (STV) and spatial (transmural, intraventricular and interventricular) dispersion[21] through the enhancement of BVR and spatial heterogeneity, respectively (Fig. 1A). Detailed mapping studies performed in the CAVB dog model revealed the importance of focal activity, resulting from afterdepolarization-related triggered activity, in the initiation and perpetuation of TdP,[21–24] whereas re-entrant circuits were less dominant.[24] Importantly, induction of TdP is highly reproducible (95%) on the administration of a proarrhythmic drug.[13]

At the cellular level, afterdepolarizations are termed early (EADs) or delayed (DADs), depending on their timing relative to the action potential. EADs arise during phase 2 (plateau) or phase 3 (repolarization) of the action potential and can be generated through the reactivation of the L-type calcium channel under conditions of prolonged repolarization. In addition, calcium overload of the sarcoplasmic reticulum[25] may lead to spontaneous calcium sparks through the ryanodine receptors and generate an EAD through the inward current created by the reverse mode of the sodium–calcium exchanger (NCX).[25] DADs occur once repolarization is complete and are mediated by the NCX in a similar mechanism to that described for EADs. Both EADs and DADs have been demonstrated as the mechanisms initiating arrhythmogenesis in the CAVB dog model.[26]

ANTIARRHYTHMIC STRATEGIES STUDIED IN THE CAVB DOG MODEL

In the present overview, antiarrhythmic strategies investigated in vivo are classified as pharmacological and nonpharmacological interventions. Associated effects on repolarization parameters are summarized in Tables 1 and 2, respectively. In addition, changes in heart rate (RR interval) can be found in **Supplemental Digital Content 1** (see **Tables 1** and **2**, http://links.lww.com/JCVP/A261).

Pharmacological Interventions

In the CAVB dog, after a baseline period, pharmacological antiarrhythmic interventions were evaluated using 2 different protocols: after (suppression experiment) and/or preceding (prevention experiment) a dofetilide or sertindole proarrhythmic challenge (Fig. 1B). In suppression experiments, antiarrhythmic drugs were only administered in dogs exhibiting TdP in a reproducible manner on proarrhythmic challenge. Because TdP reproducibility between experiments is approximately 95% in CAVB dogs,[13] prevention experiments were performed only in dogs inducible in a previous experiment. Drugs were ranked according to their ability to suppress or prevent incidence of TdP in CAVB dogs: high (<20%), moderate (20%–80%), or low/absent (>80% of dogs with remaining TdPs) antiarrhythmic effect. A TdP arrhythmia was defined as a ventricular tachycardia of at least 5 consecutive ectopic beats characterized by twisting QRS morphologies around the isoelectric line. For the periods corresponding to baseline, proarrhythmic challenge and antiarrhythmic drug, an arrhythmia score (AS) was calculated as the average of the most 3 severe arrhythmic events within 10 minutes (Fig. 1B): no ectopic beat (no EB: 1 point), single EB (sEB: 2 points), multiple EB (mEB: 3–5 points), beats of TdP (6–50 points), and number of defibrillations (50, 75, and 100 points for 1, 2 or ≥3 defibrillations, respectively). Statistical analysis of AS was performed using a nonparametric paired test (Friedman test). *P* values lower than 0.05 were considered significant. Electrophysiological parameters presented in this overview are represented by QT interval corrected for heart rate [QTc, using van de Water formula: $QTc = QT - 0.087 \times (1000 - RR)$], left and right ventricular endocardial monophasic action potential duration (LV and RV MAPD), STV derived from LV MAPD and calculated from 30 consecutive beats to assess BVR as previously described by Thomsen et al[14] ($STV = \Sigma|D_{n+1} + D_n - 2D_{mean}|/[30 \times \sqrt{2}]$) to assess BVR, and interventricular dispersion of repolarization (ΔMAPD = LV−RV MAPD) as a surrogate for spatial dispersion of repolarization.

Drugs With High Antiarrhythmic Efficacy

Calcium blockers flunarizine (2 mg/kg) and verapamil (0.4 mg/kg) demonstrated a very robust antiarrhythmic effect in CAVB dogs by completely suppressing dofetilide-induced TdP[27] and reducing AS to baseline. This strong antiarrhythmic effect was associated with the restoration of STV values to baseline levels (Table 1 and Fig. 2A). Although sharing a similar antiarrhythmic profile, the effect on QTc and interventricular dispersion of repolarization (ΔMAPD) greatly differed: flunarizine reduced both parameters to baseline values, whereas verapamil did not decrease them (Table 1 and Fig. 2A). In prevention experiments, flunarizine and verapamil did not provoke any arrhythmias. In addition, both drugs also successfully prevented the incidence of dofetilide-induced TdP[27] and kept AS low by significantly limiting the STV increase associated with dofetilide challenge (Table 1 and Fig. 2B), despite the considerable and significant prolongation of repolarization including QTc (Table 1). Interestingly, administration of flunarizine, but not verapamil, resulted in the reduction of STV and shortened repolarization duration compared with baseline (Table 1 and Fig. 2B). Reactivation of L-type calcium current occupies a central role in the incidence of EADs. Therefore, inhibition of this current results in an efficient antiarrhythmic effect. Additional cellular investigations showed that flunarizine also inhibited the late sodium current (late I_{Na}), whereas verapamil reduced the frequency of calcium sparks during diastole. Enhancement of these 2 components is known to reduce repolarization reserve and to contribute significantly to the generation of afterdepolarizations.[28,29] These additional blocking properties certainly contribute to the high antiarrhythmic efficacy of flunarizine and verapamil.

Although highly efficient against ventricular arrhythmias, calcium antagonists produce a significant negative inotropic

Table 1. Suppressive and Preventive Antiarrhythmic Efficacy in CAVB Dogs Sensitive to Drug-Induced TdP Arrhythmias

	Dose	TdP Incidence After Drug	QTc, milliseconds			LV MAPD, milliseconds		
			Baseline	Challenge	Drug	Baseline	Challenge	Drug
Suppression experiment								
Flunarizine (n = 10)	2 mg/kg	0/10*	421 ± 49	553 ± 40†	425 ± 38*	355 ± 35	492 ± 53†	367 ± 42*
Verapamil (n = 7)	0.4 mg/kg	0/7*	424 ± 62	566 ± 87†	516 ± 90†	349 ± 88	505 ± 110†	466 ± 95†
SEA0400 $ (n = 4)	0.8 mg/kg	0/4	408 ± 51	549 ± 95	702 ± 45	—	—	—
Levcromakalim (n = 7)	3 μg/kg	2/7	367 ± 50	460 ± 69†	481 ± 120†	330 ± 44	428 ± 78†	410 ± 99†
	10 μg/kg	1/7*	—	—	380 ± 44*	—	—	323 ± 66*
Lidocaine (n = 6)	3 mg/kg	2/6	360 ± 51	489 ± 41	503 ± 72	328 ± 23	478 ± 38†	394 ± 63
Ranolazine (n = 5)	4 mg/kg, +0.225 mg·kg^{-1}·min^{-1}	2/5	416 ± 59	523 ± 69†	489 ± 88†	361 ± 59	538 ± 80†	497 ± 81†
W7 (n = 6)	50 μmol/kg	2/6	421 ± 75	569 ± 137†	467 ± 94*	354 ± 54	520 ± 111†	408 ± 151*
K201								
low dose (n = 3)	0.1 mg/kg, +0.01 mg·kg^{-1}·min^{-1}	8/8	435 ± 68	550 ± 96†	nq	333 ± 59	483 ± 103†	nq
high dose (n = 5)	0.3 mg/kg, +0.03 mg·kg^{-1}·min^{-1}							
AVE0118 (n = 3)	0.5 mg/kg	3/3	—	—	—	362 ± 25	498 ± 40†	380 ± 50†

	ΔMAPD, milliseconds			STV, milliseconds			AS			Reference
	Baseline	Challenge	Drug	Baseline	Challenge	Drug	Baseline	Challenge	Drug	
Suppression experiment										
Flunarizine (n = 10)	51 ± 28	97 ± 56†	48 ± 32*	1.8 ± 0.5	4.5 ± 1.5†	1.5 ± 0.6*	1.0 ± 0.0	50.8 ± 13.9†	1.4 ± 0.8*	27
Verapamil (n = 7)	44 ± 39	72 ± 36	92 ± 92	1.7 ± 0.4	3.2 ± 1.1†	1.5 ± 0.7*	1.1 ± 0.4	71.2 ± 13.4†	1.9 ± 0.8*	27
SEA0400 $ (n = 4)	—	—	—	6.2 ± 3.7	12.0 ± 6.4	7.3 ± 3.2	1.0 ± 0.0	63.9 ± 6.8†	7.2 ± 10.2	30
Levcromakalim (n = 7)	25 ± 20	55 ± 46	52 ± 73	1.8 ± 0.5	4.9 ± 2.1†	2.6 ± 0.9*	1.2 ± 0.3	29.8 ± 16.9†	4.5 ± 3.6	17
	—	—	18 ± 52	—	—	2.0 ± 0.4*	—	—	2.0 ± 0.8	
Lidocaine (n = 6)	43 ± 21	120 ± 64	73 ± 36	1.5 ± 0.2	3.6 ± 0.8†	2.3 ± 0.9*	1.4 ± 0.6	37.1 ± 28.8†	6.6 ± 7.1	15
Ranolazine (n = 5)	33 ± 14	53 ± 34	63 ± 19	2.5 ± 0.4	4.5 ± 0.8†	3.2 ± 0.5*	1.3 ± 0.4	35.9 ± 21.7†	16.1 ± 19.3	15
W7 (n = 6)	—	—	—	2.1 ± 0.9	3.1 ± 1.1	2.7 ± 1.6	1.2 ± 0.4	47.7 ± 25.9†	5.9 ± 8.6	33
K201										
low dose (n = 3)	—	—	—	1.5 ± 0.5	3.1 ± 0.5†	nq	1.6 ± 0.8	47.6 ± 19.8†	31.5 ± 27.4	34
high dose (n = 5)										
AVE0118 (n = 3)	—	—	—	2.3 ± 0.9	5.3 ± 0.1†	nq	1.0 ± 0.0	43.1 ± 6.2†	27.0 ± 12.3	35

	Dose	TdP Incidence Challenge	QTc, milliseconds			LV MAPD, milliseconds		
			Baseline	Drug	Challenge	Baseline	Drug	Challenge
Prevention experiment								
Flunarizine (n = 8)	2 mg/kg	0/8	413 ± 51	369 ± 41†	476 ± 77†*	299 ± 44	277 ± 36	380 ± 65†*
Verapamil (n = 6)	0.4 mg/kg	0/6	417 ± 58	417 ± 41	611 ± 34†*	332 ± 68	328 ± 34	554 ± 77†*
Lidocaine (n = 6)	3 mg/kg	3/6	382 ± 34	318 ± 20†	586 ± 38†*	323 ± 41	281 ± 25	522 ± 51†*
Ranolazine (n = 6)	4 mg/kg, +0.225 mg·kg^{-1}·min^{-1}	4/6	392 ± 38	438 ± 43	565 ± 31†*	362 ± 49	410 ± 49	573 ± 56†*
K201								
Low dose (n = 6)	0.1 mg/kg, +0.01 mg·kg^{-1}·min^{-1}	5/6	400 ± 50	467 ± 55†	—	309 ± 45	386 ± 62	438 ± 123†
High dose (n = 3)	0.3 mg/kg, +0.03 mg·kg^{-1}·min^{-1}	3/3	410 ± 61	493 ± 53†	—	338 ± 51	458 ± 78†	368 ± 62
AVE0118								
Low dose (n = 6)	3 mg/kg	6/6	438 ± 47	438 ± 44	500 ± 74†*	384 ± 49	398 ± 64	481 ± 103†
High dose (n = 2)	10 mg/kg	2/2	376 ± 27	360 ± 48	—	344 ± 74	342 ± 73	—

	ΔMAPD, milliseconds			STV, milliseconds			AS			Reference
	Baseline	Drug	Challenge	Baseline	Drug	Challenge	Baseline	Drug	Challenge	
Prevention experiment										
Flunarizine (n = 8)	42 ± 27	22 ± 16	38 ± 42	1.5 ± 0.6	1.0 ± 0.5†	1.4 ± 0.5	1.6 ± 0.9	1.4 ± 0.6	1.6 ± 0.5	27
Verapamil (n = 6)	32 ± 29	33 ± 16	30 ± 16	1.3 ± 0.4	1.4 ± 0.6	2.3 ± 1.4	1.0 ± 0.0	1.3 ± 0.7	2.3 ± 1.3	27
Lidocaine (n = 6)	53 ± 29	36 ± 11	169 ± 44†*	1.4 ± 0.3	1.2 ± 0.2	3.1 ± 0.4†*	1.0 ± 0.0	1.0 ± 0.0	9.7 ± 13.0	15
Ranolazine (n = 6)	53 ± 14	47 ± 15	73 ± 26	1.6 ± 0.3	2.0 ± 0.3	3.3 ± 0.9	1.1 ± 0.1	1.3 ± 0.4	18.4 ± 22.0	15

(*continued on next page*)

Table 1. (*Continued*) Suppressive and Preventive Antiarrhythmic Efficacy in CAVB Dogs Sensitive to Drug-Induced TdP Arrhythmias

	ΔMAPD, milliseconds			STV, milliseconds			AS			
	Baseline	Drug	Challenge	Baseline	Drug	Challenge	Baseline	Drug	Challenge	Reference
K201										
Low dose (n = 6)	36 ± 29	74 ± 49†	—	1.0 ± 0.5	1.3 ± 0.7	3.3 ± 0.7†*	1.1 ± 0.2	1.5 ± 1.1	21.7 ± 24.6†	34
High dose (n = 3)	48 ± 40	98 ± 61†	—	1.2 ± 0.3	2.9 ± 0.8†	3.1 ± 1.8†	1.3 ± 0.6	2.0 ± 1.2	29.4 ± 33.5†	
AVE0118										
Low dose (n = 6)	49 ± 31	64 ± 26	103 ± 70	2.1 ± 0.4	2.1 ± 0.3	4.6 ± 1.8†*	1.4 ± 1.3	1.3 ± 0.6	41.4 ± 18.4	35
High dose (n = 2)	59 ± 38	58 ± 12	—	2.5 ± 0.1	2.5 ± 0.1	—				

TdP incidence is determined as number of dogs with TdP/number of experiments.
Values are represented as mean ± SD.
*$p<0.05$ versus Challenge (suppression) or versus Drug (prevention).
†$p<0.05$ versus Baseline.
$, STV was determined from QT interval; ΔMAPD, interventricular dispersion of repolarization (difference LV−RV MAPD); nq, not quantifiable; QTc, QT interval corrected for heart rate [van de Water formula, QTc = QT−0.087 × (RR−1000)]; STV of repolarization (derived from LV MAPD).

effect,[30] which prohibits their use in patients with heart failure. In an attempt to preserve cardiac contractile function, NCX inhibition appeared as an interesting pharmacological strategy while providing efficient antiarrhythmic properties. In the CAVB dog model, the NCX inhibitor SEA0400 (0.8 mg/kg) suppressed all TdP arrhythmias induced by dofetilide.[30] Although further prolongation of repolarization was observed after SEA0400 (Table 1), the antiarrhythmic effect was associated with a reduction of STV after SEA0400, despite not reaching statistical significance (Table 1). Importantly, a comparative study between verapamil and SEA0400 showed that the NCX inhibitor, unlike the calcium antagonist, did not evoke negative inotropy while exhibiting a comparable antiarrhythmic effect.[30]

Administered after sertindole-induced TdP, the 2 consecutive doses (3 and 10 μg/kg) of the adenosine triphosphate sensitive potassium current ($I_{K,ATP}$) opener levcromakalim decreased the incidence of TdP in a dose-dependent manner, from 6/7 dogs after sertindole to 2 and 1/7 dogs after low and high dose of levcromakalim, respectively.[17] Infusion of the low dose of levcromakalim was accompanied by a significant decrease in STV, whereas repolarization parameters such as QTc and ΔMAPD remained unaffected (Table 1). The high dose, however, shortened repolarization duration and reduced STV back to baseline values (Table 1 and Fig. 2A).

Hence, the high antiarrhythmic effect of this group of drugs is associated with a decrease in STV back to baseline level (suppression) or a reduced increase in STV after dofetilide (prevention) independent of the changes in QTc and ΔMAPD.

Drugs With Moderate Antiarrhythmic Efficacy

Antiarrhythmic drugs showing moderate efficacy in the CAVB dog model include drugs with different pharmacological mechanisms. Although most of these drugs were studied in a suppression regimen, only the I_{Na} inhibitors lidocaine and ranolazine have been additionally tested in prevention experiments. The late I_{Na}, enhanced in patients with heart failure, prolongs repolarization and reduces repolarization reserve, facilitating afterdepolarizations to occur in these patients.[28] Administered after dofetilide-induced TdP, lidocaine and ranolazine reduced TdP incidence to 2/6 and to 2/5 dogs, respectively,[15] and not significantly reduced AS. Likewise, a modest (although significant) STV reduction was observed with lidocaine and ranolazine (Table 1 and Fig. 2A). These higher STV values in comparison with the drug from the previous category were in accordance with the remaining proarrhythmic activity after administration of both I_{Na} inhibitors. In prevention experiments, lidocaine and ranolazine similarly limited incidence of dofetilide-induced TdP, reflected by the reduced magnitude of STV increase (Table 1 and Fig. 2B). The reduction of I_{Na} density in CAVB left ventricular myocytes might explain the limited antiarrhythmic efficacy of lidocaine and ranolazine, which could not fully compensate for the downregulation and acute inhibition of I_{Kr}.[15]

Calcium–calmodulin protein kinase II (CaMKII), a protein playing a central role in calcium handling, is elevated in patients with heart failure.[31,32] In the CAVB dog model, dofetilide acutely increases CaMKII activity before the occurrence of TdP.[33] Modulation of CaMKII activity by the calmodulin inhibitor W7 was partially effective and resulted in the suppression of TdP in 4/6 CAVB dogs. This antiarrhythmic effect was accompanied by a significant shortening of QTc (Table 1). Despite inducing TdP, dofetilide administration did not result in a significant increase of STV (Table 1). Therefore, W7 only mildly reduced STV values (Table 1).[33]

In summary, the decrease in STV and AS in the suppression experiments did not reach baseline values, but again these antiarrhythmic effects were accompanied by no clear reduction in QTc and/or ΔMAPD. In prevention experiments, lidocaine was responsible for a less pronounced increase in STV after dofetilide, whereas QTc and ΔMAPD showed large increases.

Drugs With Low or No Antiarrhythmic Efficacy

Among the pharmacological antiarrhythmic interventions tested in the CAVB dog model, K201 and AVE0118 have shown a low or absent antiarrhythmic potential to suppress or prevent drug-induced TdP arrhythmias (Table 1). K201 is a multichannel blocking ($I_{K,Ach}$, I_{Kr}, I_{Na}, I_{CaL}, and I_{K1}) drug, which mainly stabilizes ryanodine receptors, therefore limiting the incidence of EADs and DADs by prevention of calcium sparks.[34] AVE0118, an atrial-specific agent inhibiting

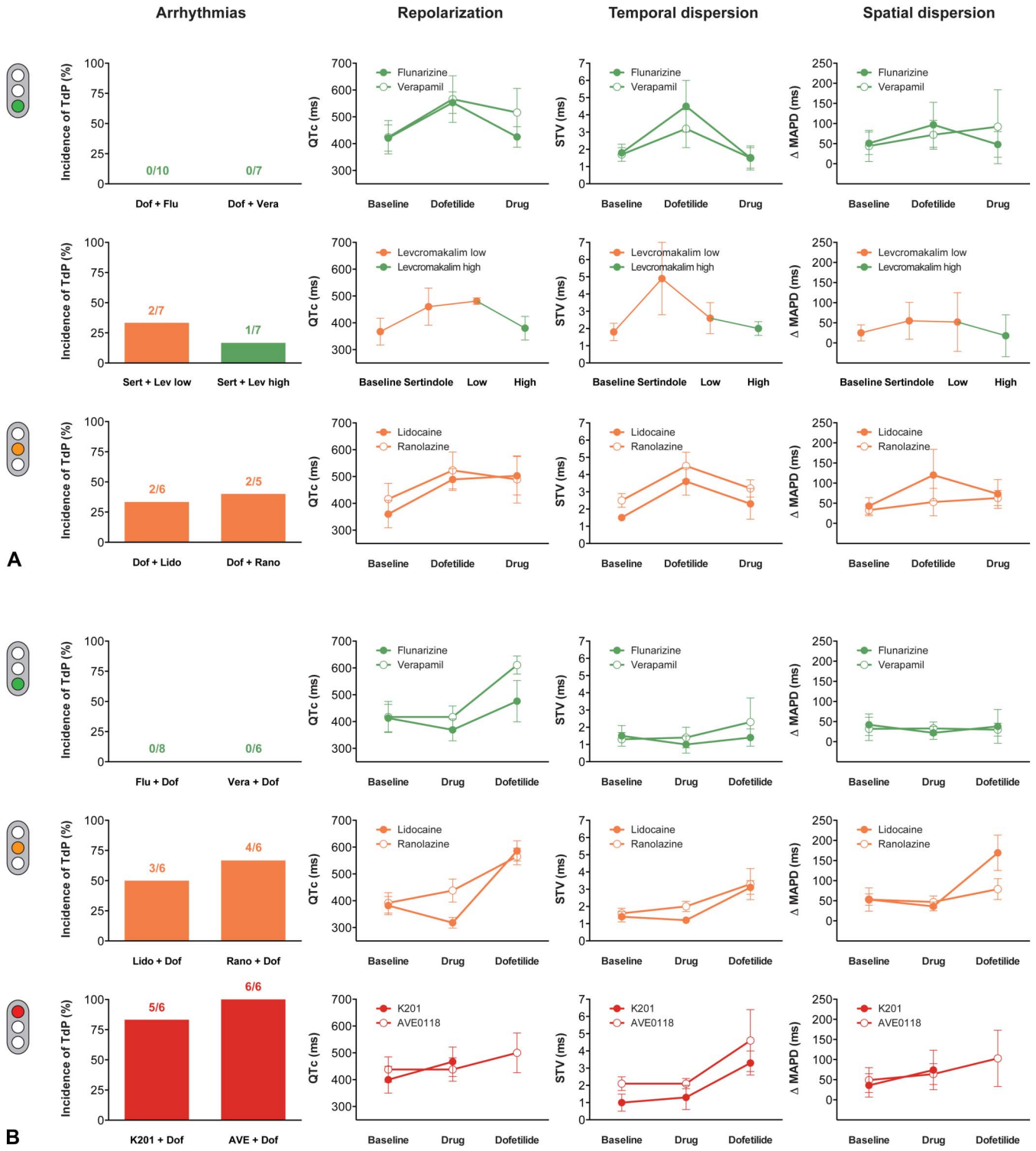

FIGURE 2. Temporal dispersion of repolarization (STV) is superior to repolarization (QTc) and its spatial (interventricular ΔMAPD) dispersion parameters in reflecting the magnitude of antiarrhythmic effect in suppression (A) and prevention (B) experiments against TdP arrhythmias. Moderate antiarrhythmic effect by low-dose levcromakalim was accompanied by the reduction of STV but not of other repolarization parameters (QTc and ΔMAPD). Subsequent administration of high-dose levcromakalim exerted stronger antiarrhythmic activity associated with a further STV reduction. Arrhythmias are plotted as percentage (number of TdP observed/number of experiments). Electrophysiological parameters: values are represented as mean ± SD. QTc, QT corrected for heart rate (van de Water formula); STV of repolarization (derived from LV MAPD); ΔMAPD, interventricular dispersion of repolarization (determined as LV−RV MAPD).

the potassium currents I_{Kur} and I_{KAch}, prolongs atrial but not ventricular repolarization.[35] Based on its pharmacological profile, it could have been anticipated that AVE0118 would not have antiarrhythmic properties against dofetilide-induced arrhythmias. Although STV could not be measured in suppression experiments because of the remaining ectopic activity, administration of dofetilide after either K201 or AVE0118 pretreatments considerably increased STV in the prevention experiments (Table 1 and Fig. 2B).

Thus, when antiarrhythmic effect was low or even absent, determination of electrophysiological parameters was difficult, especially in suppression experiments. The increase in STV in the prevention experiments clearly indicated no antiarrhythmic action.

Nonpharmacological Interventions

Elevation of potassium (K^+) by K^+ chloride pretreatment (4.6 ± 0.9 vs. 2.7 ± 0.4 mM in the control experiment, $P < 0.05$) prevented sertindole-induced TdP, in which incidence dropped from 6/7 (control experiment) to 1/7 dogs (with potassium pretreatment, Table 2).[17] This highly preventive antiarrhythmic effect was associated with the reduction of sertindole-induced increase in STV (Table 2), whereas repolarization parameters were prolonged to a similar extent in both experiments, such as LV MAPD (Table 2).

Moreover, this study also showed that acute ventricular pacing (1000 milliseconds cycle length) after sertindole-induced TdP under idioventricular rhythm exerted complete suppression of ventricular arrhythmias, which was associated with the significant reduction of STV but not by repolarization parameters including LV MAPD (Table 2).[17] Another study proved that high-rate pacing (600 milliseconds cycle length) could significantly prevent dofetilide-induced TdP arrhythmias incidence in dogs previously known to be susceptible at a lower rate (1000 milliseconds cycle length). This was associated with the reduction of dofetilide-induced increase in STV but not in repolarization parameters (Table 2).[20]

EVALUATION OF ANTIARRHYTHMIC AGENTS IN ISOLATED SINGLE CARDIOMYOCYTES

Whole-cell patch clamp technique using the current clamp configuration is a method often used in isolated cardiomyocytes to determine drug effects on repolarization and their potential proarrhythmic risk liability. Similar to in vivo experiments, BVR is involved in arrhythmic susceptibility at the cellular level. The increase in STV was previously shown to predict, beyond the extent of action potential duration (APD) prolongation, proarrhythmic events (incidence of EADs) in isolated cardiomyocytes (Fig. 1C).[14] Conversely, antiarrhythmic drugs are also evaluated using this in vitro method to gain more insight about their electrophysiological and antiarrhythmic effects.

As observed in CAVB dogs, class III antiarrhythmic challenge (dofetilide 1 μM) resulted in APD prolongation of canine ventricular cardiomyocytes associated with an increase in STV before the occurrence of EADs (Fig. 1C and Table 3). The successful suppression of EADs after administration of an antiarrhythmic drug was, similarly as in vivo, associated with the reduction in STV instead of repolarization (APD)

TABLE 2. Antiarrhythmic Efficacy of Nonpharmacological Interventions in CAVB Dogs

			QTc, milliseconds		LV MAPD, milliseconds	
	Conditions	TdP Incidence Challenge	Baseline	Challenge	Baseline	Challenge
Control	$[K^+]$ = 2.7 ± 0.4 mM	6/7	351 ± 61	439 ± 64*	324 ± 55	408 ± 65*
Potassium pretreatment	$[K^+]$ = 4.6 ± 0.9 mM*	1/7†	376 ± 66	457 ± 101*	345 ± 53	432 ± 72*
Control	IVR	5/6	—	448 ± 70	—	414 ± 78
Ventricular pacing	PCL 1000 milliseconds	0/6†	—	491 ± 83	—	403 ± 114
Control (low-rate pacing)	PCL 1000 milliseconds	6/7	445 ± 24	534 ± 69*	320 ± 28	427 ± 97*
High-rate pacing	PCL 600 milliseconds	1/7	435 ± 23	501 ± 78*	251 ± 16†	296 ± 59†

	ΔMAPD, milliseconds		STV, milliseconds		
	Baseline	Challenge	Baseline	Challenge	Reference
Control	16 ± 18	37 ± 43	2.0 ± 0.7	4.5 ± 1.3*	17
Potassium pretreatment	38 ± 27	65 ± 44	2.2 ± 0.7	3.0 ± 1.1†	
Control	—	45 ± 36	—	4.9 ± 1.5	17
Ventricular pacing	—	52 ± 45	—	3.2 ± 1.0†	
Control (low-rate pacing)	—	—	1.7 ± 0.6	3.0 ± 1.8*	20
High-rate pacing	—	—	0.9 ± 0.2	1.5 ± 1.4	

TdP incidence is determined as number of dogs with TdP/number of experiments.
Values are represented as mean ± SD.
*$P < 0.05$ versus baseline.
†$P < 0.05$ versus control.
$[K^+]$, kalemia; ΔMAPD, interventricular dispersion of repolarization (difference LV−RV MAPD); IVR, idioventricular rhythm; PCL, pacing cycle length; QTc, QT interval corrected for heart rate (van de Water formula); STV, short-term variability of repolarization (derived from LV MAPD).

TABLE 3. Suppressive Antiarrhythmic Efficacy in Isolated Canine Ventricular Cardiomyocytes

	Concentration	EAD Incidence After Drug	APD, milliseconds			STV, milliseconds			Reference
			Baseline	Challenge	Drug	Baseline	Challenge	Drug	
Flunarizine (n = 8)	1 μM	0/8*	337 ± 119	507 ± 153†	289 ± 60†	14 ± 14	65 ± 34†	11 ± 5†	27
SEA0400 (n = 11)	1 μM	0/11*	352 ± 66	764 ± 160†	680 ± 171†	8 ± 4	65 ± 10†	20 ± 6†	30
Ranolazine (n = 9)	15 μM	0/9*	436 ± 98	526 ± 125†	486 ± 94	17 ± 8	34 ± 16†	19 ± 8	15
W7 (n = 6)	50 μM	0/6*	479 ± 98	594 ± 233†	352 ± 35†	40 ± 27	70 ± 54†	16 ± 9†	33
KN93 (n = 9)	50 μM	1/9*	405 ± 113	752 ± 356†	463 ± 133	18 ± 11	91 ± 43†	35 ± 18†	33
Cyclosporin (n = 4)	1 mg/mL	3/4	340 ± 70	541 ± 118†	nq	12 ± 8	77 ± 65†	nq	33

EAD incidence is determined as number of cells with EAD/number of experiments.
Values are represented as mean ± SD.
*$P < 0.05$ versus challenge.
†$P < 0.05$ versus baseline.
APD, action potential duration; nq, not quantifiable; STV, short-term variability of repolarization (derived from APD).

shortening (Table 3). As an example, exposition to SEA0400 (1 μM) resulted in the complete suppression of EADs in 11 cardiomyocytes.[30] This strong antiarrhythmic effect was associated with a reduction of STV from 65 ± 10 milliseconds after dofetilide to 20 ± 6 milliseconds after SEA0400 ($P < 0.05$), despite APD remaining prolonged (Table 3).

The comparison of outcomes collected from in vivo and in vitro models revealed a higher sensitivity of isolated cardiomyocytes in response to antiarrhythmic drugs. For example, ranolazine or W7, although both moderately effective in CAVB dogs (Table 1), exhibited a strong antiarrhythmic effect in vitro (Table 3). Several reasons may explain these differences. First, isolated cardiomyocytes tested in patch clamp experiments are highly likely to be exposed to a higher drug concentration than the unbound drug fraction reaching the cardiac tissue in vivo, subsequently resulting in a different degree of interaction with ion channels (on and off targets). Second, the nature of arrhythmic episodes observed in both models differs greatly. Despite being initiated by a triggered activity mechanism, TdP arrhythmias in vivo require cell-to-cell coupling and a three-dimensional component (spatial heterogeneity) combined with other modulators such as mechanical workload or autonomic nervous system. These factors are all absent in the simplified in vitro system, in which only EADs can be induced through a single (vs. multiple in-vivo) hit on repolarization.[36] Moreover, the use of isolated cardiomyocytes has shown to lack specificity in recognizing safe drugs in cellular proarrhythmic screening.[37,38] Nevertheless, these cellular experiments remain complementary to the in vivo model by providing a useful approach to gain insight into the underlying antiarrhythmic mechanisms.

COMPARING THE DIFFERENT METHODOLOGIES TO ASSESS ANTIARRHYTHMIC EFFICACY

In general, the suppression and prevention experiments revealed similar results. All drugs tested belonged to a certain category, independent to the methodology chosen. It is also clear that isolated myocytes demonstrate a more optimal picture in classifying drug antiarrhythmic actions: more drugs belonged to the high antiarrhythmic category as seen in vivo.

STV OF REPOLARIZATION IS SUPERIOR TO REPOLARIZATION PARAMETERS IN PREDICTING ANTIARRHYTHMIC EFFECT

As mentioned before, the administration of an I_{Kr} blocker in CAVB dogs always results in repolarization prolongation (Fig. 1A). However, although the extent of repolarization delay cannot discriminate between resistant and susceptible dogs to TdP, STV is the only parameter able to accurately predict the incidence of ventricular arrhythmias.[14,39] Therefore, STV may offer the possibility to individually evaluate the proarrhythmic susceptibility in relation to repolarization reserve, after ventricular remodeling and/or acute proarrhythmic challenge.[12]

Moreover, the observations from antiarrhythmic interventions studied in the CAVB dog model and in isolated canine cardiomyocytes have demonstrated that STV is also superior to other repolarization parameters in reflecting their respective antiarrhythmic efficacy. The successful intervention of suppressing TdP incidence was always accompanied by the reduction in STV value, regardless of the evolution of other repolarization parameters (Table 1 and Fig. 2, eg, flunarizine vs. verapamil). In the case of a highly effective antiarrhythmic effect, STV returned to baseline values, whereas STV in the moderately effective drugs was only mildly (but significantly) reduced. Therefore, we hypothesize that successful suppressive therapy, terminating arrhythmic episodes, is able to acutely increase the repolarization reserve associated with STV reduction. In the prevention setting, both flunarizine and verapamil maintained a high repolarization reserve, which prohibited dofetilide-induced increase in STV and associated ectopic activity (Table 1).

The significant antiarrhythmic efficacy of verapamil, levcromakalim (low dose) (Table 1), and ventricular pacing (Table 2) was only associated with a reduction of STV without a shortening in repolarization. Moreover, despite the significant QTc increase after dofetilide challenge in verapamil pretreated dogs, the absence of arrhythmic events was, once more, accompanied by the low STV value (Table 1). These findings further support the use of STV reduction, instead of repolarization shortening, as a strong indicator for antiarrhythmic efficacy.

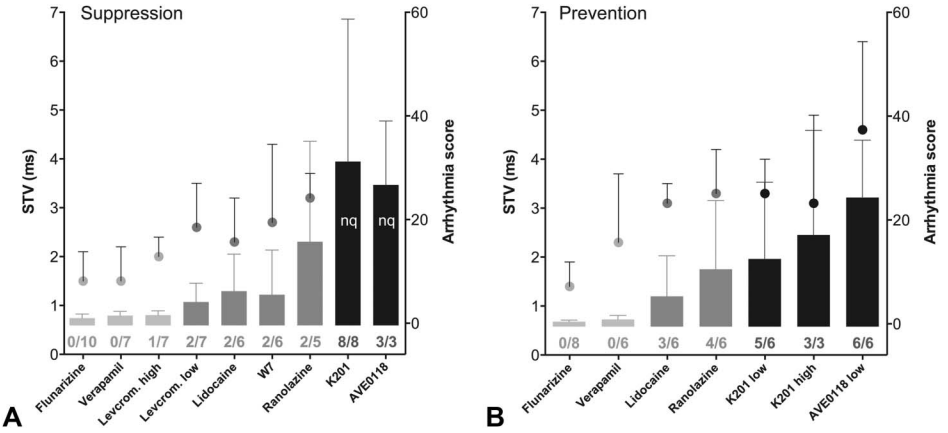

FIGURE 3. STV of repolarization strongly indicates the pharmacological antiarrhythmic efficacy in suppression (A) and prevention (B) experiments. STV values (dots), depicted for suppression (after antiarrhythmic drug administration) and prevention (after dofetilide administration) experiments are represented as mean ± SD. AS (bars) is expressed as mean ± SD. TdP inducibility is indicated as number of dogs with TdP/number of experiments. nq: not quantifiable. STV, short-term variability of repolarization (derived from LV MAPD).

In suppression experiments, the level of STV after the pharmacological intervention seemed to positively correlate with its associated antiarrhythmic efficacy (Fig. 3A). Likewise, in the prevention regimen, the extent of dofetilide-induced STV increase in pretreated animals was strongly associated with the protective effect of the antiarrhythmic agent (Fig. 3B). Therefore, this suggests that STV could be used as a strong indicator for antiarrhythmic drug evaluation. In addition, along with QTc interval, changes in the spatial (interventricular, ΔMAPD) dispersion of repolarization were not always associated with the observed antiarrhythmic outcome. Generally, the administration of a proarrhythmic challenge (dofetilide or sertindole) resulted in ΔMAPD increase thereby supporting previous preclinical and clinical data, which together showed that conditions of increased spatial dispersion of repolarization favored incidence of ventricular arrhythmias in long QT syndrome.[40–43] However, unlike STV, the increase of ΔMAPD lacked specificity in discriminating inducible from resistant CAVB dogs.[39] Recently, a detailed mapping study investigating the mechanism of TdP in the CAVB dog model confirmed that interventricular difference in repolarization was not a determinant factor for TdP inducibility.[21] Moreover, it revealed that the focus of ectopic activity constantly arose from the site of maximal heterogeneity of repolarization. Importantly, the increased local (intraventricular and transmural) heterogeneity of repolarization was found in dogs susceptible to TdP and also correlated with arrhythmic event severity, demonstrating that a minimum local gradient of repolarization was necessary for the initiation of a TdP.[21] In addition to the present observations, these findings suggest that ΔMAPD may not be specific for antiarrhythmic evaluation because this parameter does not capture the local change in repolarization heterogeneity.

For an antiarrhythmic intervention to be efficient in the CAVB dog model, there are, in principle, several possibilities (Fig. 1A, underlined items): a complete reversal of the abnormal repolarization and its temporal and spatial dispersion components (eg, flunarizine) or the reduction of temporal dispersion with minor effects on repolarization (eg, verapamil). The third possibility is the reduction of spatial (intraventricular and transmural) dispersion of repolarization, a mechanism already described in relation with antiarrhythmic drugs,[40] but none has ever been investigated thus far by the group of Vos.

LIMITATIONS

Despite showing a clear correlation with the antiarrhythmic effect observed, the major drawback of STV is related to its calculation based on consecutive normal beats. Sometimes in suppression experiments, the low antiarrhythmic potency of the drug tested, as observed with K201 and AVE0118, makes the determination of STV impossible because of the residual ectopic activity. Nevertheless, the prevention setting, which provided comparable outcomes in antiarrhythmic efficacy to the suppression setting, may offer an elegant alternative to circumvent this problem.

In this comparison, only interventricular dispersion of repolarization has been used to quantify the relevance of spatial dispersion. We are well aware of other methods of quantification, such as at the intraventricular or transmural level, which may provide additional information.

In this animal model, antiarrhythmic efficacy of interventions was evaluated along with associated electrophysiological changes in an acute manner after or preceding a proarrhythmic challenge. The evaluation of impact on survival is clearly beyond the scope of this model and remains to be investigated further by follow-up studies in patients.

CONCLUSIONS

The shortening of repolarization or the reduction in interventricular dispersion of repolarization does not appear to be most important when trying to quantify antiarrhythmic efficacy of pharmacological and nonpharmacological interventions in the CAVB dog model. STV was the only parameter to predict the magnitude of efficacy of different antiarrhythmic drugs against class III–induced TdP in vivo in both suppression and prevention experiments, as well as against EADs in vitro.

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